Resveratrol protects against diabetes-induced endothelial dysfunction in high-fat diet fed mice

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Epidemiological studies have shown that red wine consumption is associated with reduced in cardiovascular mortality in the general population and diabetic patients. The present study was designed to investigate whether resveratrol (RSV, a red wine antioxidant) can attenuate diabetes progression, improves diabetes-related vascular endothelial dysfunctions, and delineated further its underlying mechanisms.

Male C57Bl/6 mice were fed with high-fat diet (HFD) for 17 weeks. Animals developed type 2 diabetes characterized by elevated body weight, hyperglycemia, hyperinsulinemia, and hyperlipidemia. Oral gavage fed with RSV (5 or 10 mg/kg/day for 17 weeks) significantly reduced body weight, plasma glucose, insulin, and triglyceride compared to non-treated HFD mice. RSV treatment also attenuated plasma glucose elevation and improved insulin responses during glucose tolerance test. Furthermore, HFD diabetic mice exhibited an increasing the numbers of leukocyte rolling, adhering, and transmigrating in the post-capillary venules of cremaster muscle. In contrast, treatment of RSV significantly attenuated leukocyte rolling, adhesion, and transmigration in HFD diabetic mice. The phenylephrine (PE)-induced vasoconstriction was dramatically attenuated in HFD diabetic mice; whereas, RSV treatment significantly improved the vessel responsiveness to PE. Our result also shows that the phosphorylated AMPK (5’AMP-activated protein kinase), Akt, and eNOS (endothelial nitric oxide synthase) protein levels were significantly reduced in aorta of HFD mice. Consistence with the observation on improvement of blood vessel responsiveness, RSV also elevated AMPK, Akt, and eNOS protein phosphorylation levels. Taking together, these results indicate that RSV attenuated diabetes-related vascular endothelial dysfunctions, at least in part, by elevation of AMPK, Akt, and eNOS proteins phosphorylation.
RESVERATROL PROTECTS AGAINST DIABETES-INDUCED ENDOTHELIAL DYSFUNCTION IN HIGH-FAT DIET FED MICE

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ABSTRACT

Epidemiological studies have shown that red wine consumption is associated with reduced cardiovascular mortality in the general population and diabetic patients. The present study was designed to investigate whether resveratrol (RSV), a red wine antioxidant, can ameliorate diabetes progression, improves diabetes-related vascular endothelial dysfunctions, and delineated further its underlying mechanisms.

Male C57BL/6 mice were fed with high-fat diet (HFD) for 17 weeks. Animals developed type 2 diabetes characterized by elevated body weight, hyperglycemia, hyperinsulinemia, and hyperlipidemia. Oral gavage fed with RSV (5 or 10 mg/kg/day for 17 weeks) significantly reduced body weight, plasma glucose, insulin, and triglyceride compared to non-treated HFD mice. RSV treatment also attenuated plasma glucose elevation and improved insulin responses during glucose tolerance test. Furthermore, HFD diabetic mice exhibited an increasing numbers of leukocyte rolling, adhering, and transmigrating in the post-capillary venules of cremaster muscle. In contrast, treatment of RSV significantly attenuated leukocyte rolling, adhesion, and transmigration in HFD diabetic mice. The phenylephrine (PE)-induced vasconstriction was dramatically attenuated in HFD diabetic mice whereas, RSV treatment significantly improved the vessel responsiveness to PE. Our result also shows that the phosphorylated AMPK (5'-AMP-activated protein kinase), Akt, and eNOS (endothelial nitric oxide synthase) protein levels were significantly reduced in aorta of HFD mice. Consistency with the observation on improvement of blood vessel responsiveness, RSV also elevated AMPK, Akt, and eNOS protein phosphorylation levels. Taking together, these results indicate that RSV attenuated diabetes-related vascular endothelial dysfunctions, at least in part, by elevation of AMPK, Akt, and eNOS protein phosphorylation.

RESULT

Table 1. Metabolic characteristics in mice fed with chow (C), or with high fat diet for 17 weeks (HFD) the HFD mice either treated with RSV 5 or 10 mg/kg for 17 weeks (HRS or HR10).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>CR</th>
<th>HFD</th>
<th>HR5</th>
<th>HR10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>26.43±0.31</td>
<td>24.20±0.33***</td>
<td>36.93±1.28***</td>
<td>31.87±1.12†</td>
<td>28.93±1.17††</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>4.39±0.10</td>
<td>3.39±0.16**</td>
<td>2.38±0.13**</td>
<td>2.73±0.18†</td>
<td>2.75±0.25†</td>
</tr>
<tr>
<td>Calories intake (kcal)</td>
<td>11.6±0.34</td>
<td>11.6±0.56**</td>
<td>10.9±0.58**</td>
<td>12.57±0.81†</td>
<td>12.6±1.15†</td>
</tr>
<tr>
<td>Water intake (ml)</td>
<td>4.29±0.04</td>
<td>3.75±0.38**</td>
<td>2.04±0.37**</td>
<td>2.73±0.48†</td>
<td>2.45±0.20†</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>96.40±2.71</td>
<td>72.20±2.06**</td>
<td>131.60±6.55**</td>
<td>109.80±1.78†</td>
<td>106.41±5.1†</td>
</tr>
<tr>
<td>Plasma insulin (mg/l)</td>
<td>0.55±0.02</td>
<td>0.64±0.02*</td>
<td>0.67±0.04*</td>
<td>0.72±0.02†</td>
<td>0.68±0.03†</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>55.91±7.77</td>
<td>47.03±1.94**</td>
<td>363.36±6.69**</td>
<td>156.00±3.72</td>
<td>129.70±0.67††</td>
</tr>
<tr>
<td>Plasma triglyceride (mg/dl)</td>
<td>65.64±1.88</td>
<td>61.31±3.24</td>
<td>127.42±13.32***</td>
<td>108.78±10.93</td>
<td>66.54±12.38††</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE*, vs. C †, vs. HFD, †, p<0.05; ††, p<0.01.

Figure 1. Resveratrol improved the glucose tolerance (A) in both chow (C) and high fat diet (HFD) fed mice. Values are expressed as mean±SE*, p<0.05, C vs. CR, †, HFD vs. C; ††, HFD vs. HR 5, †, HFD vs. HR 10. (B) Area under curve. Values are expressed as mean±SE*, vs. C, †, vs. HFD, †, p<0.05; ††, p<0.01.

Figure 2. Leukocyte rolling, adhesion, and transmigration in post-capillary venules of cremaster muscle were evaluated in control (C), HFD, HFD+RSV 5 or 10mg/kg (HR 5 or 10). The numbers of rolling, adhering, and transmigrating leukocytes per high-power field were counted over a 1-minute period. Values are expressed as mean±SE*, vs. C †, vs. HFD, †, p<0.05; ††, p<0.01.

Figure 3. Vascular reactivity in response to variety of vasodilators or vasoconstrictors in artery and vein. Ach, acetylcholine; SNP, sodium nitroprusside; PE, phenylephrine. Values are expressed as mean±SE*, p<0.05, C vs. CR, †, HFD vs. C; †, HFD vs. HR 5; †, HFD vs. HR 10.

Figure 4. Morphology and structure of the aorta of mice fed with chow (C), or with high fat diet for 17 weeks (HFD) the HFD mice either treated with RSV 5 or 10mg/kg for 17 weeks (HR5 or HR10). Values are expressed as mean±SE*, vs. C †, vs. HFD, †, p<0.05.

Figure 5. Nitric oxide level of plasma in mice fed with chow (C), or with high fat diet for 17 weeks (HFD) the HFD mice either treated with RSV 5 or 10mg/kg for 17 weeks (HR5 or HR10). Values are expressed as mean±SE*, vs. C †, vs. HFD, †, p<0.05.

Figure 6. Endothelial glucose transporter 1 (GLUT1). CuZn-SOD and MnSOD protein levels in mice fed with chow (C), or with high fat diet for 17 weeks (HFD) the HFD mice either treated with RSV 5 or 10 mg/kg for 17 weeks (HR5 or HR10). Values are expressed as mean±SE*, vs. C †, vs. HFD, †, p<0.05; ††, p<0.01.

Conclusion

RSV
Resveratrol
Inhibition of diabetes-induced hyperglycemia
Inhibition of diabete-induced insulin resistance
Inhibition of diabetes-induced hyperlipidemia
Inhibition of diabetes-induced hyperuricemia
Inhibition of diabetes-induced hyperuricemia
Inhibition of diabetes-induced hyperuricemia
Inhibition of diabetes-induced hyperuricemia
Inhibition of diabetes-induced hyperuricemia
Inhibition of diabetes-induced hyperuricemia

Obesity
Hyperuricemia
Hyperinsulinemia
Impaired glucose tolerance

Vascular dysfunction
Leukocyte/endothelium adhesive interaction
Vascular reactivity decreased
Vascular morphology changed

Figure 9. Endothelial AMPK and phospho-AMPK protein levels in mice fed with chow (C), or with high fat diet for 17 weeks (HFD) the HFD mice either treated with RSV 5 or 10 mg/kg for 17 weeks (HR5 or HR10). Values are expressed as mean±SE*, vs. C †, vs. HFD, †, p<0.05; ††, p<0.01.